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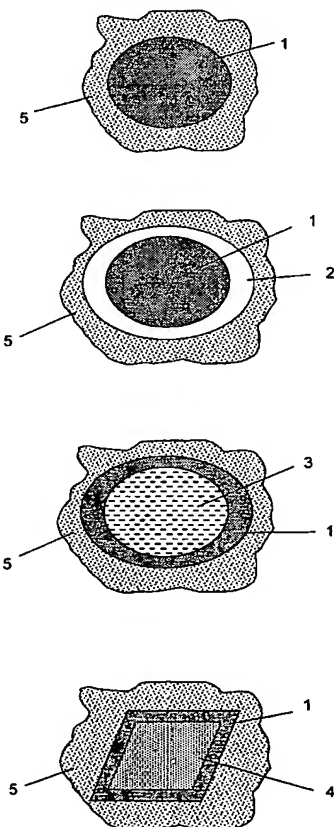
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(54) Title: DELIVERY OF NEUTRON CAPTURE ELEMENTS FOR NEUTRON CAPTURE THERAPY



(57) Abstract: Neutron capture therapy (NCT) for example, Boron neutron capture therapy (BNCT) requires the delivery of a neutron capture element such as Boron to a target site to be treated, followed by irradiation with neutrons. The invention provides new means for delivery of the neutron capture element in the form of insoluble inorganic nanoparticles having a particle size of about 10^{-10} m to about 10^{-6} m. The neutron capture element can be in particulate form or in the form of glass or glass ceramic or as a polymerised inorganic matrix or as a sol-gel derived xerogel. The nanoparticles of the invention can further comprise a biocompatible outer layer which provides the function of stealth and assists in providing an appropriate clearance rate. In some embodiments, the nanoparticles comprise a core selected from, for example, mica, zeolites, TiO_2 spheres, ZrO_2 spheres or particles or organic polymer particles or spheres surrounded by a thin film of the neutron capture element. Pharmaceutical compositions, uses and methods for the treatment of cancer are disclosed. Also disclosed is a process for the preparation of water insoluble nanoparticles comprising causing friction between pure blocks of the required neutron capture element in an inorganic form and collecting nanoparticles that result therefrom.

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DELIVERY OF NEUTRON CAPTURE ELEMENTS FOR NEUTRON CAPTURE THERAPY

5 The invention relates to neutron capture therapy and, in particular, to new ways of delivering neutron capture elements to a desired target site.

10 The theoretical principle of neutron capture therapy (NCT) for the treatment for cancer was first described in 1936 by the American scientist Locker. In essence, when a stable isotope such as Boron-10 is irradiated by non-ionizing slow neutrons, a fission reaction results that leads to the emission of highly ionizing radiation with a range of between 7 μ m and 9 μ m. Thus, when ^{10}B is irradiated in this way inside e.g. a tumour cell, the range of the resulting ionising radiation is sufficient to destroy that cell but not surrounding tissue. In 1951 the first patient was treated. Since then the principle has been
15 successfully demonstrated in the clinic.

The level of success has varied and has been dependent on a combination of the quality of neutrons and the pharmaceutical properties of the neutron capture element carrier.

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NCT is a bimodal therapy requiring spatial and temporal overlap of neutrons and the drug. To achieve a biological effect, an interaction between slow neutrons and e.g. a boron carrying agent are necessary. The NCT reaction of ^{10}B with neutrons (BNCT) may be summarised by the following equation:

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The 2.4 MeV energy is taken up as kinetic energy by the Li^+ and He^{2+} ions. The two particles are sufficiently energetic to generate intense ionization
30 tracks with a maximum range of 9 μ m. In this way, the damage is confined to the diameter of a tumour cell i.e. about 10 μ m. Thus, only the cells that contain boron are damaged, while non-boron containing healthy cells are left intact. The isotope Boron-10 is stable and non-radioactive and its nucleus

has a very large neutron absorption cross section for slow neutrons, 2,700 times greater than that for hydrogen. This translates into the ability to absorb neutrons of comparatively several thousand times better than that of the elements constituting living tissues (such as hydrogen, oxygen, carbon).

5

NCT can be used to treat cancers which are normally treated with radiotherapy such as lymphomas and skin cancers, as well as cancers of the brain, breast, lung, head and neck, bone, prostate, pancreas and cervix. In addition when surgical removal of a tumour is planned, NCT may also be used to help shrink the size of the tumour and to reduce the associated normal tissue loss.

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NCT has been driven by the potential benefits of selective "in situ" radiotherapy and as a potential substitute to conventional x-ray radiotherapy. Over 500 patients have received experimental BNCT treatment for brain and skin tumours World-wide to date.

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NCT is advantageous over conventional radiotherapy in a number of respects. Thus, in conventional radiotherapy the biological effect is spread over the entire irradiated area whilst with NCT it is specific to those cells containing neutron capture element carrier molecules. A relatively high radiation dose is required with radiotherapy to generate the destructive ionisation tracks for the biological effect. This is a limiting factor in the effectiveness of the treatment. Radiotherapy is further limited by the inherent nature of the low LET (linear energy transfer) radiation beam which comprises electrons (β -particles) or photons (X-rays and γ -rays). With BNCT short range high energy α alpha and ^7Li particles are generated. These particles are high LET particles that are intensely ionizing and exhibit a greater, more potent, destructive propensity. Hence, the BNCT dose required to generate an equivalent radiotherapy biological effect is very much smaller than the relative radiotherapy dose.

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Radiotherapy is widely used in the management and treatment of cancers where it exhibits a 30% cure rate expected to increase to 33% over 10 years, with 70% palliation. However, radiotherapy results in radiation damage to normal tissue and typically requires a 6 week treatment schedule.

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In contrast to radiotherapy NCT offers target tissue selectivity via the specificity of the drug. Lethal radiation is only generated where the neutron capture element is localised i.e. within the tumour tissue. Thus, for example, in BNCT the radiation is generated in boron containing tumour cells, and damage is localised to those cells. This is a key advantage of NCT over radiotherapy to potentially destroy tumours that are normally not clearly demarcated, but are diffuse.

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NCT is not dependent up on oxygen levels in the tumour, and this is an advantage as many tumours are hypoxic. Moreover, NCT can also be used where the cancer treatment is anatomically compromised. Deep seated tumours can be treated, for example, from 4cm-8cm depth and beyond. Additionally, NCT is less demanding for the patient than conventional radiotherapy as it can be given several times over a period of 2-4 days. By contrast, conventional radiotherapy needs to be given up to 30 times over a period of six weeks.

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A further advantage of NCT is that in such a binary system each component can be manipulated independently of the other. The interval between administration of the, e.g. ^{10}B agent and neutron irradiation can be adjusted to an optimum time when there is the highest differential ^{10}B concentrations between normal tissues and the tumour tissue. Similarly, the neutron beam can be collimated so that the field of irradiation is limited to the tumour site, and normal tissues with a residual ^{10}B concentration can be excluded from the treatment volumes.

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In summary the benefits of NCT are as follows:

- NCT is a biomodal therapy -time interval between administration of drug and the time of "drug-activation" can be optimised to attain maximum tumour kill
- Selective targeting of tumour cells and sparing of normal tissues
- 5 • Radiation generated and localised only in those cells with sufficient quantity of the neutron capture element carrying agent
- Effective for diffuse spread and potential to target distant single tumour cells or metastases
- NCT is applicable to radioresistant tumours and anatomically
- 10 compromised sites
- NCT is not dependent on oxygen content in tumours
- NCT can access over 4cm - 8cm of tumour depth, there is effectively no limitations to the depth of a tumour
- Treatment time of 2-4 days compared to typical 6 weeks with
- 15 radiotherapy

To reap the potential benefits of NCT as an "in situ" cellular radiotherapy it is a preferable that:

- 20 (i) The neutrons are of an energy range where only the neutron capture element atoms are able to undergo a fission reaction;
- (ii) The, e.g. boron atoms or the NCT agent is selectively localised in the target tissue;
- 25 (iii) For BNCT, there is optimal concentration of Boron drug in the target tissue. For example 15µg-35µg / g of tumour tissue (equating to 10^9 boron atoms) is widely quoted to be the amount of ^{10}B necessary for BNCT using a neutron beam from a nuclear reactor of fluence 10^9
- 30 neutrons per second per cm^2 ;

(iv) The neutron capture element drug should have an unusually low toxicity compared to conventional pharmaceutical agents since large concentration of the compound need to be administered; and

- 5 (v) Sufficiently rapid clearance from the blood so that there is a very high ratio of tumour to blood concentration, and a very high tumour to normal tissue concentration.

To date, predominantly two experimental compounds, 4-
10 dihydroxyborylphenylalanine (BPA) and sodium
mercaptoundecahydrododecaborate (BSH) have been used to deliver Boron
as the neutron capture element in clinical trials.

The following table (Table 1) shows some characteristics of Boron neutron
15 capture therapy agents that are currently being evaluated.

Table 1

Compound	Absolute tumour concentration (ug/g)	Tumour:blood ratio	Tumour:CNS Ratio
BPA (in clinical trials)	16.5	2.2: 1.0	1.7 :1.0
BSH (in clinical trials)	6.9	0.7 : 1.0	3.0: 1.0
CuTCPH (preclinical development)	114.0	570 : 1.0	57 : 1.0

As noted above, a requirement of NCT is that the neutron capture element is
20 targeted specifically or predominantly to the tissue site of interest. This
mission critical criterion has yet to be fully satisfied by compounds in clinical
trials to date, due to the lack of specificity and low target tissue to normal
tissue distribution ratios observed. As a result the clinical development and
wide spread use of BNCT has been severely hindered. Thus far, only the

CuTCPH compound developed by Michiko Mura appears to provide adequate boron localisation.

To date neutron capture agents have been targeted to tumors by various
5 drugs. Boron has been incorporated into a huge range of drug molecules
including protein targeting systems whose toxicity, pharmacokinetics,
biodistribution and radiobiology have been studied, and in some promising
instances attempts have been made to optimise these characteristics via
10 formulation in co-solvents and drug delivery systems. However, from over 50
years of research and development effort only two sub-optimal compounds
(BPA and BSH) have entered clinical trials. These compounds have low
toxicity but suffer from poor selectivity for tumors and inadequate
pharmacokinetics suitable for routine BNCT therapy. A more detailed review
of Boron drug candidates studied in the prior art is set out in Table 2.

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Table 2

	Boron drug candidate	Issue	Reference
1.	Boronated amino acids Eg. BPA Boronophenylalanine	Boronated phenylalanine is in clinical trials. It is not selective and the kinetics are such that it need to be given several times over a periods of day. A major problem is that it needs to be given in large volumes such as 1,000ml by intravenous injection. This causes haemodynamic disturbance which poorly tolerated by patients whom are already	Snyder H R et al (1958) J Am Chem Soc 80, 835.

	Boronated amino acids and peptides	seriously ill.	See pages 1531-1534 Soloway (1998) Chem Rev.
2.	Modified carborane cage Eg. $[B_{10}H_{10}]^{2-}$ decahydrodecaborate Eg. $[B_{12}H_{12}]^{2-}$ Dodecahydrodecaborate Eg. $C_2B_{12}H_{12}$	Not very effective as standalone but are used as boron carriers for attachment to other molecules.	Hawthorn, M F et al (1959) J Am Chem Soc 81, 5519. Grimes R, N. Carboranes. Academic press. NY 1970.
3.	$B_{12}H_{12} SH^{2-}$ Mercaptoundecahydrododecaborate (BSH) May also be in the form or a dimer "BSSB" Is a second generation BNCT compound	In clinical trials, not very selective, inappropriate kinetics, several doses need to be given.	Soloway A H et al J Med Chem 1967, 10. 714.
5	Porphyrins and Phthalocyanines Eg. BOPP,	Porphyrins carry 4 carborane cages. BOPP as been shown to have phototoxicity and poor selectivity for tumours.	Ozawa T., Pro Am Ass for Cancer March 1998, 39, p586

	Eg. CuTCPH	CuTCPH appears to show favorable tumour :blood: normal tissue distribution.	Miura M.2001, Radiation research 155, 603-610.
6.	<p>Boron containing Nucleic acid precursors</p> <p>Boronated pyrimidines and purines</p> <p>5-(dihydroxyboryl) uracil</p> <p>5-carboranyluracil</p> <p>carborane containing pyrimidines</p> <p>(these are considered as third generation compounds)</p>	Targeted as precursors for DNA and RNA synthesis, failed to show any promise.	<p>Liao. T.K, J Am Chem Soc 1964, 86. 1869.</p> <p>Schinazi R, F J Org. Chem. Soc 1985, 50, 841.</p> <p>Nemoto. H. J Chem Soc. Chem Commun. 1994, 577.</p>
7.	<p>Proteins</p> <p>Antibody -boron conjugates</p>	A two step bispecific antibody system is described where the boron is attached to the antibody via boronated dextran.	Griffiths G L et al. US patent 5,846,741 (1998)
8.	<p>DNA binders</p> <p>Alkylating agents, intercalators -acridines</p>	Have been synthesised but none has yet shown any promise.	Soloway 1998 Chemical reviews Vol 98 No.4 p1538-1541.

	and tetracycline's, phenanthridine Groove binders -carborane derivatives		
9.	Gd-157 agent a combined BNCT and GdNCT compound	The Gd and a carborane cage are on the same molecule.	Soloway 1998 Chemical reviews Vol 98 No.4 p1519.
9.	Lipids and Liposomes B-lipiodol	Used for encapsulating lipid soluble and water soluble compounds, BNCT compound delivery systems.	Soloway 1998 Chemical reviews Vol 98 No.4 p1533-1534. Shelly K Proc Natl Acad Sci USA 1992, 89. 9039. Chou FI, Anticancer Res 1999 May-Jun;19(3A):1759-64
10	Others including, foliates growth factors, hormones, radiation sensitisers, phosphates, phosphonates and phosphoramidates, cyclic thiourea derivatives, amines, promazines, hyantoinis, barbiturates	Many classes of these compounds have been synthesised and none have so far made it to the clinic.	Soloway 1998 Chemical reviews Vol 98 No.4 p1545-1550.

All of the above are conventional drug candidates or biopharmaceuticals that are dissolved in either an aqueous solution and/or in an organic co-solvent system that uses standard pharmaceutical excipients such as oils, alcohol's, ethers or lipids. These formulations are then administered via routes such as oral, intravenous, intraperitoneal, intramuscular, dermal and colonic. Almost always these dissolved compounds and on occasion co-solvents have shown adverse systemic toxicity and pharmacological/ metabolic breakdown activity profile (anaphylaxis, pain at the site of injection, emboli formation, and precipitation). The drug candidate molecules have one or more ^{10}B atoms incorporated into their structure via covalent bonding. Normally, carborane cages are used to add as many boron's as is possible to the central "carrier" molecule.

There is a need for improved neutron capture element carriers capable of selectively delivering the neutron capture element (for example, boron) to a target tissue. Such carriers should exhibit low toxicity and appropriate clearance kinetics, and ideally should be capable of the delivery of large doses of neutron capture elements to tumour tissue. The current invention sets out to provide such a carrier.

Thus, in a first aspect, the invention provides water insoluble nanoparticles comprising at least one neutron capture element in an inorganic form for use in therapy, surgery or diagnosis. In a second aspect, the invention provides a pharmaceutical composition comprising a water insoluble nanoparticle comprising at least one neutron capture element in an inorganic form.

Any form of nanoparticle may be used in the invention, as long as it is water insoluble. Thus, although the term nanoparticle is sometimes used to describe colloidal particles consisting of macromolecular substances (whether artificial or natural), and often includes liposomes, proteins, peptides, strands of DNA and RNA, and polymers, only those nanoparticles that are water insoluble are intended to be covered. Thus, the present invention utilises

materials that are insoluble in aqueous media and, in general, in all conventional pharmaceutical solvents.

- The at least one neutron capture element is present in the water insoluble nanoparticle in an inorganic form. The neutron capture element is preferably boron and, in particular, ^{10}B , but may be any one or more of the elements selected from the group consisting of ^6Li , ^{22}Na , ^{22}Co , ^{113}Co , ^{126}I , ^{135}Xe , $^{148\text{m}}\text{Pm}$, ^{149}Sm , ^{151}Eu , ^{155}Gd , ^{157}Gd , ^{164}Dy , ^{184}Os , ^{199}Hg , ^{230}Pa , ^{235}U , ^{241}Pu .
- 10 The neutron capture element may be present in a natural crystalline form, or in a particulate form. Alternatively, the neutron capture element may be in the form of a glass or glass ceramic, in the form of a polymerised inorganic matrix or in the form of a sol-gel derived xerogel. In some embodiments, the neutron capture element may also be in the form of an organically modified ceramic,
- 15 for example, wherein the element comprises at least one bond to a hydrocarbon chain. Such organically modified ceramics behave as inorganic materials because the metal-oxygen-metal system dominates over the few metal-carbon bonds. Such organically modified ceramics maintain the neutron capture element in a system that is inert and insoluble in water.
- 20 Nanoparticles of some embodiments of the invention may further comprise a biocompatible outer layer which can be selected from the group consisting of polymers, organic or inorganic pharmaceutical excipients, low molecular weight oligomers, natural products, for example, gelatines, gums, fatty acids,
- 25 soya bean oils or purified fractions thereof or ionic surfactants and non-ionic surfactants. Such hydrophilic biocompatible outer layers impart the function of stealth to the nanoparticles, allowing them to escape detection by the reticuloendothelial system of the liver, spleen and pancreas. The outer covering also acts as a surfactant and functions to prevent aggregation of the
- 30 particles.

These biocompatible layers are adsorbed onto the core structure and do not necessarily chemically react to the surface. Preferably, the outer layer does not include intramolecular cross-linkages.

In an alternative embodiment, the neutron capture element can be present as a layer or film around an inorganic nanoparticle core such as mica, zeolites, TiO₂ spheres, ZrO₂ spheres or particles, or organic polymer particles or
5 spheres.

In a further embodiment, nanoparticles of the invention may further comprise a pharmacologically active substance which may, or may not be loaded into the nanoparticles by absorption, adsorption or incorporation. In preferred
10 embodiments, the pharmacologically active substance is a chemotherapeutic agent.

In some embodiments, nanoparticles of the invention further comprise a metal selected from the group consisting of vanadium (V), manganese (Mn), iron
15 (Fe), ruthenium (Ru), technetium (Tc), chromium (Cr), platinum (Pt), cobalt, (Co), nickel (Ni), copper (Cu), zinc (Zn), germanium, (Ge), indium (In), tin (Sn), yttrium (Y), gold (Au), barium (Ba), tungsten (W), and gadolinium (Gd). In some embodiments, the further metal is present at a concentration of about 0.0001% wt/wt to about 0.1% wt/wt.

20 In a third aspect, the invention provides the use of water insoluble nanoparticles comprising at least one neutron capture element in an inorganic form in the manufacture of a medicament for use in neutron capture therapy. The invention also provides, in a fourth aspect, a method for neutron capture
25 therapy comprising (i) administering a water insoluble nanoparticle comprising at least one neutron capture element in an inorganic form to an individual; (ii) allowing said nanoparticles to accumulate at a desired location in the body; and (iii) administering neutrons to said individual. Additionally, the invention provides the use of water insoluble nanoparticles comprising at least one
30 neutron capture element in an inorganic form in a method for neutron capture therapy.

In some embodiments of any of the above uses or methods, the neutron capture therapy is for the treatment of ablation of cancer or other diseased

tissues, for example, lymphomas, skin cancer, breast cancer, lung cancer, head and neck cancer, bone cancer, prostate cancer, cancer of the pancreas, cervical cancer, brain cancers e.g. glioblastomas, primary and secondary metastases and benign and metastatic prostate cancers, e.g. benign prostate hyperplasia. In some embodiments, the tumours to be treated are solid and discrete. In treatment methods of the invention, neutron capture therapy can be administered over a period of e.g. 1 to 14 days. The therapeutic methods of the invention may further comprise the step of removing a tumour by surgery. In a further embodiment, the method of the invention comprises analysing the concentration of the neutron capture element in the target tissue, for example, using MRI, PET or SPECT imaging.

For dosemetry it is necessary to calculate the effective therapeutic BNCT radiation dose so as to provide the optimal treatment protocol. For this, knowledge of the NCT compound concentration in the target tissue is required. Conventionally, blood concentration or ideally the target tissue concentrations are derived using invasive techniques. The invention allows the incorporation of Magnetic Resonance Imaging MRI contrast agents such as Gadolinium or radionuclotides such as ^{67}Cu and Tc-99m-HMPAO for Positron Emission Tomography (PET) and Single photon emission computed tomography (SPECT) respectively. MRI provides vital anatomical diagnostic information in addition to the NCT compound concentration. PET, allows the examination of the heart, brain, and other organs and images show the chemical functioning of an organ or tissue and is particularly useful for the detection of cancer, coronary artery disease and brain disease, unlike X-ray, CT, or MRI which show anatomical structure. Single-photon emission computed tomography (SPECT), like PET acquires information on the concentration of radionuclotides.

In a fifth aspect, the invention provides a process for the preparation of water insoluble nanoparticles comprising at least one neutron capture element in an inorganic form, said process comprising (i) providing at least a first mass of said neutron capture element in an inorganic form; (ii) providing at least a second mass of the same type of material; (iii) mixing said first and second

masses in the absence of other abrasive material; (iv) causing frictional abrasion between said first and second masses; and (v) collecting said nanoparticles.

- 5 In some embodiments of this aspect of the invention, at least one further, non-abrasive material is included in the mixing step, and the non-abrasive material may be selected from the group consisting of vanadium (V), manganese (Mn), iron (Fe), ruthenium (Ru), technetium (Tc), chromium (Cr), platinum (Pt), cobalt, (Co), nickel (Ni), copper (Cu), zinc (Zn), germanium, (Ge), indium (In),
10 tin (Sn), yttrium (Y), gold (Au), barium (Ba), tungsten (W), and gadolinium (Gd)...

The invention is now described in more detail with reference to the following drawings in which:-

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Figure 1 shows a first and second embodiment of an insoluble inorganic nanoparticle.

Figure 2 shows a third embodiment of an insoluble inorganic nanoparticle.

Figure 3 shows a fourth embodiment of an insoluble inorganic nanoparticle.

- 20 Figure 4 shows various embodiments of insoluble inorganic nanoparticles having biocompatible outer stealth layers.

- 25 In one embodiment the invention utilizes angstrom (\AA) sized particles that are totally insoluble solid inorganic materials that may have their surface properties modified for optimal pharmacokinetics and minimal toxicity for neutron capture therapy in the treatment of, for example, various forms of cancer.

- 30 The current invention overcomes the problems of cytotoxicity and the deposition of a high amount of neutron capture element at the tumour site and minimises the amount of neutron capture element in normal tissues. The invention provides for rapid clearance from the blood and further provides a diagnostic option to image tumour location and size and to pick up distant metastases.

In a preferred embodiment, the invention involves the direct use of ^{10}B as a metal, and inorganic materials such as Boron-transition metal complexes, boron oxides, boron nitrides, boron carbides and large carbonate cages. The physical form of these materials may be angstrom sized particles or engineered angstrom sized particles which may be coated with a biocompatible surface modifier e.g. a surfactant. Alternative engineered particles comprise an organic polymer or an inorganic non-NCT element containing core covered with a ^{10}B coating and optionally covered with a biocompatible surfactant. Further alternative engineered particles comprise a ^{10}B inorganic or mostly inorganic core or a Boron containing polymer core that is covered by a chemically and mechanically protective coating, for example a metal oxide coating, that is optionally further covered with a biocompatible surfactant

15

Figure 1 shows the basic form of the inorganic, insoluble nanoparticles of some embodiments of the invention. The nanoparticle 1 consists of pure NCT metal, such as ^{10}B glass or ceramic and has a size of between about 1 and 10,000 Å (10^{-10} to 10^{-6} m). Preferably, the nanoparticles are between about 1 and 1,000 Å (10^{-10} to 10^{-7} m), more preferably between about 1 and 100 Å (10^{-10} to 10^{-8} m) and ideally between about 10 and 100 Å (10^{-9} to 10^{-8} m).

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Figure 1b shows an alternative embodiment, in which the pure NCT metal particle 1 is surrounded by a chemically and/or mechanically protective coating, 2, for example, TiO_2 . In each case, the neutron capture element of nanoparticle 1 can be in the form of, for example, a metal compound, a glass, ceramic, crystal, ormacil or xerogel. Preferably, the neutron capture element is Boron, although other elements are useful for neutron capture element as discussed above.

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The physical form of the inorganic NCT agent may be its natural crystalline form or in the form of particles, or spheres or polymeric structures. This will depend up on the manufacturing route. Physical properties of the angstrom sized nanoparticles particles can also be those typically characterised by a

Glass, (a super cooled liquid, that is an amorphous randomly organised molecular structure which does not produce an x-ray diffraction pattern), a ceramic -crystalline phase or glass-ceramic (a structure that exhibits characteristics of a glass that has a crystalline phase). Alternatively, the nanoparticles may be sol-gel derived materials (polymerised inorganic matrix ~ metal-oxygen-metal), sol-gel derived xerogels (partially or fully condensed material), or an organically modified ceramic where the metal has one or more M-R bonds where R is a hydrocarbon chain. These materials are chemically stable and totally insoluble in aqueous media although they may react with water producing oxides but do not dissolve. The NCT materials do not dissolve in water even at elevated temperatures. Silica glasses are amongst the most chemically inert of commercial materials. They react with almost no liquids or gases at low temperatures (~300C) and at higher temperatures they only react with gaseous HF. In some embodiments, nanoparticles of the invention cannot be detected after about 2 to about 12 hours in the blood plasma.

Figure 2 shows an alternative embodiment of the invention, in which the neutron capture element, for example, Boron, is present as a thin film surrounding an inorganic or organic polymer core, and Figure 3 shows a further alternative embodiment in which the neutron capture element 1 is present as a thin film surrounding an inorganic or organic crystalline core which may or may not contain the neutron capture therapy element. Neutron capture element layer 1 has a thickness of anything from a few atoms to tens of atoms, and the core can be, for example, mica, zeolites, TiO₂ spheres, ZrO₂ spheres or particles or organic polymer particles or spheres.

Figure 4 shows the various embodiments of Figures 1 to 3 modified by the addition of an outer biocompatible stealth layer 5. The biocompatible stealth layers are adsorbed onto the core structure. They do not necessarily chemically react to the surface and preferably do not have any intermolecular cross-linkage. The layers are selected from a group consisting of well known organic and inorganic pharmaceutical excipients including various polymers, low molecular weight oligomers, natural products and preferably ionic and

non-ionic surfactants. Examples of the excipients include gelatin, casein, lectine (phosphatides), gum acacia, calcium stearate, cholesterol, tragacanth, sorbitan esters, stearic acid, benzalkonium chloride, glyceryl monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, , polyoxyethylene alkyl
5 ether (e.g. macrogol ethers such as cetomacrogol 1000, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters (eg. Commercially available Tweens), polyethylene glycols, polyoxyethylene stearates, colloidal silicon dioxide, colloidal titanium dioxide, phosphates, sodium dodecylsulphate, carboxymethylcellulose calcium or sodium,
10 methylcellulose, hydroxyethylcellulose, hydroxypropylmethylcellulose phthalate, noncrystalline cellulose, hydroxypropylcellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol (PVA), and polyvinylpyrrolidone (PVP). (Handbook of Pharmaceutical Excipients by American Pharmaceutical Association and The Pharmaceutical society of
15 Great Britain, Pharmaceutical Press 1986).

The preferred biocompatible stealth layers include (I) block copolymers of ethylene oxide and propylene oxide; polyvinyl pyrrolidone, Pluronic F68 and F108; Tetronic 908, tetrafunctional block copolymer derived from the addition of
20 ethylene oxide and propylene oxide to ethylenediamine, dextran, lecithin, Carbowax 3350 and 934 (polyethylene glycols, Union Carbide), aerosol OT (dioctyl ester of sodium sulfosuccinic acid from American Cyanamide), Tween 80 (polyoxyethylene sorbitan fatty acid ester, ICI chemicals), Duponol P (sodium lauryl sulfate, Dupont), Triton X-200 (alkyl aryl polyether sulfonate,
25 Rohm and Hass).

Particularly preferred stealth layers include lectin, Pluronic and F-68 polyvinylpyrrolidone, polyoxyethylene 20 sorbitan monolaurate, polyoxyethylene 20 sorbitan monopalmitate, polyoxyethylene 20 sorbitan
30 monostearate and polyoxyethylene 20 sorbitan monooleate or mixtures of any of the above.

In some embodiments, particles of the invention may be loaded with a pharmacologically active substance by absorption, adsorption, or

incorporation to impart a dual therapeutic effect of chemotherapy and NCT.

The raw particles may be coated with a surfactant as noted above directly in the presence or in the absence of a loaded pharmaceutical agent.

Chemotherapeutic agents may be loaded to deliver a double blow to a cancer

- 5 cell in the form of an instantaneous combination therapy. Suitable chemotherapeutic agents include: melphalan, cyclophosphamide, doxorubicin, carmusline, methotrexate, 5-fluorouracil, cytarabine, mercaptopurine, anthracyclines, daunorubicin, epirubicin, vinca alkaloids, mitomycin C, vinblastin, vincristine, dactinomycin, taxol, Lasparaginase; G-CSF, cisplatin,
- 10 carboplatin.

Other drugs and their prospective prodrugs which may be incorporated into nanoparticles of some embodiments include any one of the following:

- 15 1. 5-azurudub-l-yl)-4-dihydroxylamino-2-nitrobenzamide
2. Gancyclovie triphosphate
3. Phenylenediamine mustard
4. Benzoic acidic mustards
5. Adenine arabinonucleoside triphosphate (araATP)
- 20 6. Phenylenediamine mustard
7. Hydrogen peroxide
8. Superoxide, hydrogen peroxide
9. Methotrexate
10. Phenylenediamine mustards
- 25 11. Cyanide
12. Etoposide
13. Topoisomerase inhibitors such as camptothecin and topotecan
14. Mitomycin alcohol
15. Palytoxion
- 30 16. Melphalan
17. Taxanes, such as taxol and taxotere
18. 5-(aziridin-l-yl)-4-hydroxylamino-2-nitrobenzamide
19. Actinomycin D, Mitomycin C

The insoluble nanoparticles of the invention can be formulated into pharmaceutical compositions for the treatment, by neutron capture therapy of a number of tumours and other tissue disorders. Treatment involves the administration of an effective dose to a patient, followed by irradiation with neutrons.

The determination of an effective dose is well within the capability of those skilled in the art. For any NCT compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in an appropriate animal model. The animal model is also used to achieve a desirable concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of active agent which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals (e.g., ED_{50} , the dose therapeutically effective in 50% of the population; and LD_{50} , the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD_{50}/ED_{50} .

Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage may be chosen by the individual physician in view of the patient to be treated. Dosage and administration can be adjusted to provide sufficient levels of the active moiety or to maintain the desired effect.

Additional factors which may be taken into account include the severity of the disease state (e.g. tumour size and location); age, weight and gender of the

patient; diet; time and frequency of administration; drug combination(s); reaction sensitivities; and tolerance/response to therapy.

Administration of pharmaceutical compositions of the invention may be accomplished orally or parenterally. Methods of parenteral delivery include topical, intra-arterial, intramuscular, subcutaneous, intramedullary, intrathecal, intraventricular, intravenous, intraperitoneal, or intranasal administration. In addition to the neutron capture element nanoparticles and optional active ingredients, these pharmaceutical compositions can contain suitable pharmaceutically acceptable carriers comprising excipients and other compounds that facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration can be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co, Easton PA).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, etc, suitable for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable additional compounds, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers include, but are not limited to sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; and gums including arabic and tragacanth; as well as proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

Dragee cores can be provided with suitable coatings such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterise the quantity of active compound (i.e. dosage).

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders such as lactose or starches, lubricants such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds can be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations for parenteral administration include aqueous suspensions of NCT nanoparticles of the invention. For injection, the pharmaceutical compositions of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions can contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the nanoparticles can be prepared as appropriate oily injection suspensions. Suitable lipophilic vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes.

For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention can be manufactured in substantial accordance with standard manufacturing

procedures known in the art (e.g. by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilising processes).

- 5 The neutron capture compounds of embodiments of the invention can be further characterised as follows. Thus, the compounds comprise an insoluble inorganic material or a mostly inorganic material that contains a neutron capture element selected from ^6Li , ^{10}B , ^{22}Na , ^{22}CO , ^{113}CO , ^{126}I , ^{135}Xe , $^{148\text{m}}\text{Pm}$, ^{149}Sm , ^{151}Eu , ^{155}Gd , ^{157}Gd , ^{164}Dy , ^{184}Os , ^{199}Hg , ^{230}Pa , ^{235}U , ^{241}Pu (Solway, 10 Chem Rev 1998, p1518). In a most preferred embodiment, the neutron capture element is Boron, preferably ^{10}B .

- Boron can be employed in embodiments of the invention as elemental Boron or as part of a number of compounds. Elemental Boron, atomic No. 5, atomic 15 weight 10.8 is a black solid at 298k with a density of 2460 kg/m^3 and molar volume 4.39 cm^3 . Boron is available commercially and the most common sources of Boron are tourmaline, borax $[\text{Na}_2\text{B}_4\text{O}_5(\text{OH})_4 \cdot 8\text{H}_2\text{O}]$, and kernite $[\text{Na}_2\text{B}_4\text{O}_5(\text{OH})_4 \cdot 2\text{H}_2\text{O}]$. In general properties of boron-rich solids such as binary and ternary borides (e.g. MeB_{12} , MeB_{66} ($\text{Na}, \text{Mg}, \text{Ln}$) MeB_{14} , the borides 20 of transition elements in beta-rhombohedral boron, boron carbides are structurally similar and can be used for NCT via the particulate route.

- Small amounts of high purity boron can be made through the thermal decomposition of compounds such as BBr_3 with hydrogen gas using a heated 25 tantalum wire. Results are better with hot wires at temperatures over 1000°C .

- Compounds of Boron exhibit a close relationship with respect to crystal structures because they contain B_{12} icosahedra or related aggregates of atoms. Since these icosahedra determine the electronic structure and hence 30 chemical bonding, there is a very large similarity of chemical and physical properties. Such boron compounds are classified as "boron rich solids". In essence all "boron rich solids" may be used for NCT via the particulate route.

In general properties of boron-rich solids such as binary and ternary borides (e.g. MeB_{12} , MeB_{66} (Na,Mg,Ln) MeB_{14} , the borides of transition elements in beta-rhombohedral boron and boron carbides are structurally similar and exhibit the following properties:

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- (i) high melting points 2000 - 4000 K; e.g. boron carbide melts at about 2,900 K
- (ii) great hardness: at ambient temperatures 2000 - 4500 kp/mm². Beta-rhombohedral boron is the hardest elementary crystal after diamond, and boron carbide is the third hardest solid after diamond and cubic boron nitride.
- (iii) low density e.g. boron carbide has a density of 2.5 gcm⁻³
- (iv) small thermal extension coefficient: e.g. boron carbide $\alpha = 5.73 \cdot 10^{-6} \text{ K}^{-1}$
- (v) high resistance to chemical attack, hence low corrosivity
- (vi) high neutron absorption cross section, caused by the ¹⁰B isotope (enrichment in natural B about 20 %)
- (vii) semiconducting behavior.

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Other well know inert stable borides include Aluminium Boride, Arsenic Boride, Barium Boride , Beryllium Boride, Boron Carbide, Boron Chloride , Boron Iodine Boron Nitride, Boron Oxide, Boron Zinc, Calcium Boride, Cerium Boride, Chromium Boride, Cobalt Boride, Copper Boride, Diborane Dysprosium Boride, Erbium Boride, Europium Boride, Fermium Boride, Gadolinium Boride, Gold -Boron, Hafnium Boride, Holmium Boride, Iridium Boride, Iron Boride, Lanthanum Boride, Lithium Boride, Lutetium Boride, Magnesium Boride, Manganese Boride, Molybdenum Boride, Neodimium Boride, Neptunium Boride, Nickel Boride, Niobium Boride, Osmium Boride, Palladium Boride, Promethium Boride, Praseodymium Boride, Platinum Boride, Plutonium Boride, Rhenium Boride, Rhodium Boride, Ruthenium Boride, Sulfur Boride, Samarium Boride, Scandium Borid, Silicon Boride, Silver Boride, Strontium Boride, Tantalum Boride, Terbium Boride, Technetium Boride, Thorium Boride, Titanium Boride, Thulium Boride, Tungsten Boride, Uranium Boride, Vanadium Boride, Yttrium Boride,

Ytterbium Boride, Zirconium Boride, all of which can also be used for NCT via the particulate route.

- In some preferred embodiments, nanoparticles of the invention comprise
- 5 Boron Carbides which are inert solids with a density of $2.4 \times 10^3 \text{ kg/m}^3$ and a melting point 2450°C (K A Schwetz, J Less Common Metals, 82 (1981) 37), Boron rich carbides (M Bouchacourt, J Less Common Metals, 82 (1981) 219) and other carbides including $\text{C}_2\text{Al}_3\text{B}_{48}$ (P.Peshev, J Less Common Metals, 117 (1986) 341) may also be used. In other embodiments, nanoparticles of
 - 10 the invention may comprise BN which is a very inert solid with a density of 3.48 g/cm^2 and a melting point of 2730°C . TiB_2 is an extremely hard material with a density of 4.52 g/cm^3 and may be used in some embodiments of the invention. Boron Oxide which has a density of $2.4 \times 10^3 \text{ kg/m}^3$, and ZrB_2 which has a density 6.11 g/cm^3 may also be used. In another preferred
 - 15 embodiment, nanoparticles of the invention may comprise $\text{B}_{10} \text{H}_{14}$ decaborane (14) (boron hydride) decaboron tetradecahydride), a white crystalline solid having a melting point of 213°C , and a density of 950 kg m^{-3} . Other hydrides which can be used include B_2H_6 , B_4H_{10} , B_5H_9 , B_5H_{11} , B_6H_{10} .
 - 20 In addition to the above, the neutron capture compound may take any of the following forms:
 - (i) neutron capture element as physical solid or a gel-like particle;
 - 25 (ii) $^{10}\text{B}_x\text{M}_n$ where M is a metal but can be a non metal such as Nitrogen, Carbon, Oxygen, Chlorine, Bromine or Fluorine;
 - (iii) $^{10}\text{B}_x\text{O}_n$;
 - 30 (iv) $^{10}\text{B}_x\text{H}_n$;
 - (v) $\text{R}-^{10}\text{B}_n-\text{O}_n$ where R =hydrocarbon chain or other organic chain; or
 - (vi) $(\text{X}-\text{O}-\text{X})_n$ where X is the neutron capture element.

The overall particle size of the nanoparticles of embodiments of the invention can be about 1Å to 10,000 Å (10^{-10} to 10^{-6} m), for example, 1,000 Å to 4,000 Å (10^{-7} to 4×10^{-7} m) preferably about 1Å to about 1000 Å (10^{-10} to 10^{-7} m), but
5 ideally about 10Å to about 100Å (10^{-9} to 10^{-8} m). This range of particle sizes are particularly advantages for a number of reasons. Firstly, particles of this size can be excreted by the kidney and do not remain in the body for a prolonged period of time. Moreover, the particles do not adversely precipitate out in the alveoli of the lungs.

10 Secondly, particles in this range accumulate in tumours via the "enhanced permeability retention" effect (EPR mechanism) and are able to easily permeate the leaky vasculature of a tumour and become entrapped leading to a build up of a high concentration in the intracellular space. Tumours have a
15 negative osmotic pressure, and have no lymphatic drainage. The vast majority of compounds enter into the tumour via diffusion and tend to remain in the tumour bed and move between cells through gap junctions. NCT compounds normally enter the cell by endocytosis into the cytoplasm and end up in the lysosomal compartment. It is important to note that the NCT
20 compounds need to be targeted such that one or more of the following components of a tumour cell becomes a site of selective disruption that will lead to cell death: the cell membrane, mitochondria, endoplasmic reticulum, golgi apparatus or nucleus.

25 As well as accumulating in tumour tissues, it has been found that the nanoparticles of embodiments of the invention become internalised in the tumour cells via endocytosis and bring the NCT agent close to the nucleus which can result in more effective killing of tumour cells. Additionally, particles in the 1Å-100Å (10^{-10} to 10^{-8} m) range are particularly preferred because they
30 can travel between adjacent cells through "gap junctions", thus providing an important means of distribution to non vascularised parts of the tumour. This process generates a homogeneous distribution of the neutron capture element throughout the tumour mass thereby providing for effective NCT.

For a biologically effective BNCT reaction to take place there is a minimum requirement for the number of boron atoms at the desired location. If boron is located to the outside of a cell (more precisely on the cell membrane) then there should be at least 10^9 boron atoms for an optimal reaction. If the boron atoms are located inside the cell, say in the cytoplasm then one order of magnitude less Boron atoms are required i.e. 10^8 and if the ^{10}B atoms are in the nucleus of a cell then a further one order of magnitude less ^{10}B atoms are required. This negative concentration gradient towards the cell nucleus is due to the fact that the probability of resultant "alpha and lithium" particles damaging the DNA increases with a decrease in the distance between the nucleus and the $n + ^{10}\text{B}$ reaction.

With the known compounds BPA, BSH and CuTCPH the number of boron atoms carried is 1, 10 and 40 per molecule respectively. Thus, for example, using BPA located at the cell membrane requires 10^9 molecules, reducing to 10^8 in the cytoplasm and 10^7 in the nucleus. When using CuTCPH, 2.5×10^8 molecules are required at the cell membrane, 2.5×10^7 in the cytoplasm and 2.5×10^6 in the nucleus.

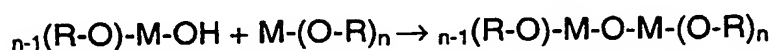
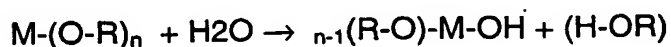
Assuming the mass of a 10 \AA spherical particle of embodiments of the invention to be $2.46 \times 10^{-24} \text{ kg}$, the number of boron atoms can be calculated based on the density of boron (2460 kg/m^3). Thus, a 10 \AA particle contains 2.28×10^{-22} moles of boron, equivalent to 140 atoms. Similarly, a 1000 \AA particle has a mass of $2.46 \times 10^{-18} \text{ kg}$ and contains 2.28×10^{-18} moles of boron, equivalent to 1.3×10^8 atoms.

In view of the above calculations it is apparent that only 7×10^7 boron particles having a diameter of 10 \AA are required to achieve an optimum BNCT reaction at the cell surface, 7×10^6 particles in the cytoplasm are required and 7×10^5 particles in the nucleus are required.

If the boron particles have a diameter of 1000 \AA then only about 1 particle is required at the nucleus or in the cytoplasm and approximately 2 particles are required at the cell surface for optimum BNCT effect.

Example 1 - Manufacture of sol-gel derived metal glass and ceramics

The sol-gel process for generating metal glass and ceramics can be described
5 as follows:



10 where M is the metal, for example Ti, Zr, Al, N, B, Si and R can be any carbon chain normally of 1-5 carbons.

This is a dynamic polymerisation reaction comprising a wet chemistry system
15 that yields a metal-oxide system from the reaction of metal-alkoxides.

The metal alkoxides are normally ethoxides, propoxides or butoxides.
Pentoxides and higher carbon chains can be used, but the reactivity drops off
with chain length and viscosity of the liquids.

20 Controlled hydrolysis is carried out with the metal alkoxides in proportions to yield a final metal oxide of given elemental ratio at room temperature in the presence of small amounts of H₂O. A fine precipitate or a gel or a xerogel can be generated via this process. By controlling the rate of hydrolysis
25 (introduction of controlled amount of moisture) a precipitate may be formed of particles of uniform size. The precipitate or gel or xerogel may be sintered at a temperature of from 100°-1000°C to drive off remaining organic residues and complete the hydrolysis and desification reactions.

30 This process is suitable for the production of Si B Glass and Ceramic systems; Si B Na Glass and Ceramic systems; Si B Na Zr Glass and Ceramic systems; Si B Na Zr Ti Glass and Ceramic systems; Ti B Glass and Ceramic

systems; Zr B Glass and ceramic systems; and Si Ti Zr B Glass and ceramic systems.

Example 2 - Manufacturing by chemical vapour deposition thin-film coatings and particles

Volatile organics such as Boron tri-ethoxide and Boron tri-isopropoxide or Boron Halogens BF_3 , BCl_3 may be used to deposit a thin film coating or generate spherical particles of the metal oxide by way of controlled introduction of moisture.

Multi-component metaloxide systems were generated using metal alkoxide precursors of Ti, Zr, Al Si, B, Na. Ethoxide, propoxide and butoxides were used to make sol-gel derived glass and glass ceramics (Titanium tetraisopropoxide, Zirconium tetraisopropoxide, Aluminium tripropoxide, Tetraethoxysilicone, Sodium ethoxide, Boron tetraethoxide, supplied by Sigma-Aldrich). All the materials except tetraethoxysilicone are hygroscopic and were handled using a vacuum line apparatus with dry nitrogen (1-3ppm water).

The tetraethoxysilicate was hydrolysed to which varying amounts of Ti, Zr, B, Na metalloids were added to produce a stable homogenous sols at room temperature.

In this way, the following metal oxide glasses were made: Si, Na B, Ti Si B, Zr Si B, Ti Zr Si B and Ti Zr Si B Na.

The above glass systems were made by first hydrolysing $\text{Si}(\text{OCH}_2\text{CH}_3)_4$ (TEOS) with ~1.5 moles of water in 0.1% HCl. Typically, 30g of TEOS, 5g of H_2O and 20ul of 3M HCl in 35g of Propanol and 35g of Ethanol was refluxed for 150 minutes. This process generates a silicate sol-gel (a partially cross linked -Si-O-Si- system) that yields between ~10% SiO_2 . To this other precursors of the metal oxide glass formers were added to generate the metal oxide glasses. Upon cooling one or more metal oxide precursors of Ti, Zr, Na,

B was gently added (3.5g of each precursor) whilst continuously stirring. A clear transparent liquid resulted. The mixture was allowed to stand at room temperature with the flask covered with cellophane with pinholes so as to allow atmospheric moisture in and for slow evaporation of solvents. The slow introduction of atmospheric moisture is necessary to aid hydrolysis of the Ti or Na or Al or Zr or B alkoxides and form a cross-linked glassy structure. After 8-24 hours standing in a fume cupboard a transparent gel results which upon further aging for up to 3-7 days shrinks in to a solid transparent xerogel (a porous glass). The xerogel was processed using mechanical pulverisation or grinding for 24-28 hours in a motorised ceramic pestle and mortar. Following this the powder was further reduced and a surfactant coating added by way of using a ball-mill.

The xerogel was heated to a temperature of 600C and 1100C to drive off any organic components leaving a black glass containing the respective metal oxides. Again the glass was processed using mechanical pulverisation or grinding for 24-28 hours in a motorised pestle mortar (25-30g grinding capacity). The powder was further reduced in particle size using the ball-mills in example 4.

These mechanical pulverisation process generated fine particles of approximately 0.1-0.5 and 1um-5um particles.

In some experiments where the reflux times were reduced to 60-120 minutes, post cooling and following the addition of metal oxide precursors of Ti, Zr, Al, B and Na a fine suspension results. The examination of the particles post evaporation of the solvents and 37 C oven drying and desiccation reveals crystalline particles of sub-micron range (typically 0.01um-1um with some in the range of 1-5um).

Example 3 – Coating nanoparticles with biocompatible "stealth" materials

At the time of particle grinding the surfaces of the particles were coated with Polyoxyethylene sorbitan fatty acid diester or Sorbitan ester of a fatty acid with

10 and 20 carbon atoms or polyvinylpyrrolidone (PVP) or Gum acacia.
Between 1% and 10% wt/wt of NCT material.

5 The surface modifier was diluted in water and a slurry (10% to 30%wt/wt) of the NCT particles was made for particle reduction in a ball-mill. The slurry was milled for 48 hours and resulted in a "milky" stable suspension. Typically, the particles were between 0.01-0.5 μ m.

Example 4 – Preparation of nanoparticles

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Boron Nitride was used to prepare nanoparticles. Boron Nitride powder of approximately 1 μ m average particle size from Aldrich, Product code 255475 was used. Polyvinylpyrrolidone K15, Molecular weight~10000, from Aldrich, Product code 81390 was used to condition the surface of the particles. The
15 mills were operated for 24-48 hours

Two mills were used to generate nanoparticles as follows:

A conventional mill constructed of porcelain having an internal diameter of 110 mm and a depth of 100 mm. This mill is run at a rotation speed of 1.25
20 Hz (35% of critical speed). The milling media was made of 10 mm diameter porcelain spheres with a total milling media volume of 500 ml. This mill was used with a mixture of, for example:

BN	7.5 g
PVP	3.0 g
25 Water	~200 ml

A customised mill has also been used. This mill was designed not to surge and be good for mixing and attrition which is the most effective mechanism for fine grinding and produces a uniform particle size. The mill has a stainless
30 steel internal diameter of 50 mm and a depth of 50 mm, and operates at a rotation speed of 1.25 Hz (14% of critical speed). The milling media was made of 3 mm diameter stainless steel spheres with a total milling media volume of approximately 50 ml. This mill may be used with a mixture of, for example:

Mixture volume (max)	~50
BN	2.50 g
PVP	0.75 g
Water	50 ml

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Example 5 – Preparation of nanoparticles by milling in the absence of abrasive

10 A conventional mill as described above was used to generate Boron Nitride nanoparticles from Boron Nitride powder, except that no milling media was used. This provided for pure Boron Nitride nanoparticles containing no contamination from milling media.

15 In a further example, the customised mill of the earlier example was also used without adding the milling media to provide pure Boron Nitride nanoparticles.

Example 6 - Experimental evaluation of inorganic nanoparticle systems for BNCT

20 **Materials and methods**

Compounds Used

25 The following methods may be used to evaluate inorganic nanoparticles of the invention, for example, comprising BN, B, BC or Si Ti B glass particles, with or without surface modification.

Surface Modification

30 The particles may be coated with the following surface modifiers to provide a biocompatible stealth layer as described above: polyoxyethylene sorbitan fatty acid ester; sorbitan ester of a fatty acid with 10-20 carbon atoms; PVA; and gum acacia.

Animal numbers and species

Studies using rodents involve standard, established techniques and statistical methods for data analysis. On average, groups of 8 rats per data point are used in pharmacokinetic, biodistribution and radiobiological studies. The animal models are male Fischer 344 rats (250-300 g), female BALB mice (20-25 g) and SCID mice (20-25 g). In all studies, anaesthesia is maintained with ketamine (120 mg/kg) and xylazine (20 mg/kg). Animals are monitored on a daily basis for general health. Animals are euthanised, as required, under anaesthesia.

Boron pharmacokinetics, biodistribution and toxicology

Evaluation of Å sized inorganic nanoparticles takes place using appropriate tumour models. These include glioma (rat 9L gliosarcoma), colon cancer (mouse), melanoma (murine Harding Passey melanoma), breast cancer (murine EMT-6 adenocarcinoma) and prostate cancer (prostate xenograft in SCID mice), all transplanted subcutaneously.

Pharmacokinetic/biodistribution analysis involve the administration of graded doses of a given boronated antibody, with sampling (blood, tumour, normal tissues) at time intervals of up to 6 days after administration. Inorganic particle systems delivering >30 µg boron/g to the tumour with a tumour:blood boron partition ratio of >3:1 is considered suitable for further evaluation. Studies also involve observations on behavior, changes in body weight, chemical and hematological tests (Miura et al., 1998).

The boron content of relevant normal tissues (skin, oral mucosa, brain, spinal cord and blood) and implanted tumours is measured using direct coupled plasma atomic emission spectrometry (Coderre et al., 1994).

Radiobiology Studies -Neutron Irradiations

Standard experimental collimators, irradiation jigs and body shielding are used for irradiations. Standard dosimetric procedures are well established and using gold foils, thermoluminescent dosimeters and ionisation chambers (Coderre et al., 1992; Morris et al., 1994a,b).

5

Pre-clinical therapeutic studies

Experimental evaluation of therapeutic efficacy entail localised irradiation of tumour-bearing animals with graded doses of thermal neutrons in the presence of the boronated antibody. The administration protocols and the timing after administration at which irradiation begins will be determined by prior pharmacokinetic and biodistribution studies. The assessment consist of quantification of changes in tumour volume, incidence of tumour ablation and long-term survival. By way of comparison, and to enable calculation of RBE/CBE factors, graded doses of x-rays and of the thermal beam alone is given.

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Histology

Standard histopathological analysis is carried out on irradiated normal tissues and tumours using standard techniques. Animals are perfusion-fixed using 10% buffered formal saline.

20

Data analysis

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Dose-response data using the various endpoints is assessed using probit analysis, and ED50 and TCP50 values are derived from the fitted curves. These values are used to calculate RBE/CBE factor values. Appropriate control groups are used in all studies. In therapy experiments, the percentage survival versus time following irradiation is analysed using the Kaplan-Meier method. The survival times in each of the experimental groups is ordered and ranked for comparison by a non-parametric statistical technique, Wilcoxin 2-Sample Test.

30

Results

In one experiment a 20%wt/wt suspension of Si Ti B glass particles coated with PVP (prepared in the ball-mill) was assessed for biodistribution as described above. An average of 10µg-25µg boron atoms per g of wet tumour tissue was found 2 days after intravenous administration.

In a further experiment, a 10%, 20% and 30% wt/wt suspension of BN particles coated with PVP were assessed for biodistribution. An average of 15µg-35µg boron atoms per g of wet tumour tissue was found 2 days after intravenous administration.

When normal tissue was measured less than 0.1 µg/g boron atoms were found in skin, whilst 1 to 5 µg/g boron atoms were found in liver and lung, demonstrating accumulation of boron preferentially in tumour tissue.

Throughout the specification, various different embodiments have been described for a number of features of the invention including, but not limited to, the physical form and size of the nanoparticles, the identity and nature of the neutron capture element and further metals to be included in the inorganic nanoparticles, uses of the nanoparticles and the presence or absence and nature of a biocompatible stealth layer as well as other ingredients in the nanoparticles. The application is intended to cover all possible combinations of each of these features and any other features provided as part of a list except where it would be apparent to the skilled person that such combinations were self-contradictory. In particular, each or any of the physical form and sizes of nanoparticles are intended to be combined individually or together with any neutron capture element in any form, and any such nanoparticles are suitable for any of the uses disclosed. Any such nanoparticle formed of any combination of the above is intended to be used with any of the biocompatible stealth layers disclosed or with no biocompatible stealth layer. Any other, and all suitable combinations will occur to the skilled reader and the Examples given are not intended to be limiting on the scope of the invention.

CLAIMS

1. A water insoluble nanoparticle comprising at least one neutron capture element in an inorganic form for use in therapy, surgery or diagnosis.
- 5 2. A water insoluble nanoparticle comprising at least one neutron capture element in an inorganic form, said nanoparticle comprising a biocompatible outer layer.
- 10 3. A water insoluble nanoparticle as claimed in claim 2 wherein said outer layer is hydrophilic.
4. A nanoparticle as claimed in any preceding claim having a particle size of about 10^{-10} m to about 10^{-6} m.
- 15 5. A nanoparticle as claimed in any preceding claim having a particle size of about 10^{-10} m to about 10^{-7} m.
6. A nanoparticle as claimed in any preceding claim having a particle size of about 10^{-9} m to about 10^{-8} m.
- 20 7. A nanoparticle as claimed in any preceding claim wherein said at least one neutron capture element is boron, preferably ^{10}B .
- 25 8. A nanoparticle as claimed in any of claims 1 to 6 wherein said at least one neutron capture element is selected from the group consisting of ^6Li , ^{22}Na , ^{22}Co , ^{113}Co , ^{126}I , ^{135}Xe , $^{148\text{m}}\text{Pm}$, ^{149}Sm , ^{151}Eu , ^{155}Gd , ^{157}Gd , ^{164}Dy , ^{184}Os , ^{199}Hg , ^{230}Pa , ^{235}U , ^{241}Pu .
- 30 9. A nanoparticle as claimed in any preceding claim wherein said neutron capture element is in its natural crystalline form.
10. A nanoparticle as claimed in any of claims 1 to 8 wherein said neutron capture element is in a particulate form.

11. A nanoparticle as claimed in any of claims 1 to 8 wherein said neutron capture element is in the form of a glass or a glass ceramic.

5 12. A nanoparticle as claimed in any of claims 1 to 8 wherein said neutron capture element is in the form of a polymerised inorganic matrix.

13. A nanoparticle as claimed in any of claims 1 to 8 wherein said neutron capture element is in the form of a sol-gel derived xerogel.

10

14. A nanoparticle as claimed in any of claims 1 to 8 wherein said neutron capture element is in the form of an organically modified ceramic and wherein the element comprises at least one bond to a hydrocarbon chain.

15 15. A nanoparticle as claimed in claim 7 wherein said boron is in the form of:

- (i) $^{10}\text{B}_x\text{M}_n$;
- (ii) $^{10}\text{B}_x\text{H}_n$; or
- (iii) $\text{R}-^{10}\text{B}_n\text{-O}_n$

20 wherein M is a metal or is selected from nitrogen, carbon, oxygen, chlorine, bromine or fluorine, x and n are integers of one or above and R is a hydrocarbon chain or other organic chain.

16. A nanoparticle as claimed in claim 7 or 8 wherein said neutron capture
25 element is in the form of $(\text{X-O-X})_n$ wherein n is an integer of 1 or above and X is the neutron capture element.

17. A nanoparticle as claimed in any of claims 1 or claims 4 to 16 further comprising a biocompatible outer layer.

30

18. A nanoparticle as claimed in any of claims 2, 3 or 17 wherein said biocompatible outer layer does not include intramolecular cross-linkages.

19. A nanoparticle as claimed in claim 17 or claim 18 wherein said biocompatible outer layer is selected from the group consisting of polymers, organic or inorganic pharmaceutical excipients, low molecular weight oligomers, natural products for example, gelatins, gums, fatty acids, soya bean oils or purified fractions thereof, ionic surfactants and non-ionic surfactants.
20. A nanoparticle as claimed in claim 18 wherein said biocompatible outer layer comprises an excipient selected from the group including gelatin, casein, lectine (phosphatides), gum acacia, calcium stearate, cholesterol, tragacanth, sorbitan esters, stearic acid, benzalkonium chloride, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, polyoxyethylene alkyl ether, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, polyoxyethylene stearates, colloidal silicon dioxide, colloidal titanium dioxide, phosphates, sodium dodecylsulphate, carboxymethylcellulose calcium or sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylmethylcellulose phthalate, noncrystalline cellulose, hydroxypropylcellulose, magnesium aluminium silicate, triethanolamine, polyvinyl alcohol (PVA), and polyvinylpyrrolidone (PVP).
21. A nanoparticle as claimed in any of claims 17 to 20 wherein said biocompatible outer layer comprises a polymer selected from:-
- (i) block copolymers, for example poly(ethylene glycol-aspartate), block copolymers of ethylene oxide and propylene oxide, tetrafunctional block copolymers derived from the addition of ethylene oxide and propylene oxide to ethylene diamine;
 - (ii) polyethylene glycol (PEG) or ethylene glycol copolymers;
 - (iii) polysaccharides, e.g. dextrin, dextran, chitosan (N-succinyl chitosan), carboxymethyl chitin, carboxymethyl pullulan or alginate;
 - (iv) poly (amino acids), for example, poly [N-(2-hydroxyethyl)-L-glutamine] PHEG, β -poly(2-hydroxyethyl aspartamide) PHEA, poly(glutamic acid), polyaspartic acid, polylysine (poly(L-lysine);
 - (v) polyesters, for example poly (α or β -malic acid);
 - (vi) alternating polymers, for example, PEG-lysine;

- (vii) copolymers of styrene and maleic anhydride;
- (viii) polygalacturonic acid;
- (ix) copolymers of hydroxalkyl(meth)acrylate e.g. N-phenylpyrrolidone, poly (L-glutamic acid and hydroxyethyl-L-glutamine), poly (α -malic acid),
5 polyaspartic acid-PEG copolymers, poly-L- lysine and copolymers of polyethyleneimine;
- (x) poly(α -L-glutamic acid) (PGA);
- (xi) biodegradable diamido-diamine polymer; and
- (xii) N-(2-hydroxypropyl) methacrylamide (HPMA copolymer)

10

22. A nanoparticle as claimed in any of claims 17 to 19 wherein said biocompatible outer layer comprises a surfactant selected from aerosol OT (dioctyl ester of sodium sulfosuccinic acid), polyoxyethylene sorbitan fatty acid ester, sodium lauryl sulfate, polyoxyethylene 20 sorbitan monolaurate,
15 polyoxyethylene 20 sorbitan monopalmitate, polyoxyethylene 20 sorbitan monostearate, polyoxyethylene 20 sorbitan monooleate and lecithin N-(2-hydroxypropyl).

20

23. A nanoparticle as claimed in any preceding claim wherein the neutron capture element is present as a layer or film around an inorganic nanoparticle core.

25

24. A nanoparticle as claimed in claim 23 wherein said core is selected from mica, zeolites, TiO_2 spheres, ZrO_2 spheres or particles or organic polymer particles or spheres.

25. A nanoparticle as claimed in any preceding claim wherein said particles further comprise a pharmacologically active substance.

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26. A nanoparticle as claimed in claim 25 wherein said pharmacologically active substance is loaded into said nanoparticles by absorption, adsorption or incorporation.

27. A nanoparticle as claimed in claim 25 or claim 26 wherein said pharmacologically active substance is a chemotherapeutic agent.

28. A nanoparticle as claimed in any preceding claim further comprising a further metal selected from the group consisting of vanadium (V), manganese (Mn), iron (Fe), ruthenium (Ru), technetium (Tc), chromium (Cr), platinum (Pt), cobalt, (Co), nickel (Ni), copper (Cu), zinc (Zn), germanium, (Ge), indium (In), tin (Sn), yttrium (Y), gold (Au), barium (Ba), tungsten (W), and gadolinium (Gd).

29. A nanoparticle as claimed in claim 28 wherein said further metal is present at a concentration of about 0.0001% wt/wt to about 0.1% wt/wt.

30. A pharmaceutical composition comprising a water insoluble nanoparticle comprising at least one neutron capture element in an inorganic form.

31. A pharmaceutical composition as claimed in claim 30 comprising a water insoluble nanoparticle according to any of claims 2 to 29.

32. The use of water insoluble nanoparticles comprising at least one neutron capture element in an inorganic form in the manufacture of a medicament for use in neutron capture therapy.

33. A use as claimed in claim 32 wherein said neutron capture therapy is for the treatment or ablation of cancer or other diseased tissues e.g. lymphomas, skin cancer, breast cancer, lung cancer, head and neck cancer, bone cancer, prostate cancer, cancer of the pancreas, cervical cancer, brain cancers e.g. glioblastomas, primary and secondary metastases and benign and metastatic prostate cancers, e.g. benign prostate hyperplasia.

34. A use as claimed in claim 33 wherein said tumour is solid and discrete.

35. A use as claimed in any of claims 32 to 34 wherein said neutron capture therapy is administered over a period of one to fourteen days.

5 36. A use as claimed in any of claims 32 to 35 wherein said nanoparticle is a nanoparticle according to any of claims 2 to 29.

37. A method for neutron capture therapy comprising:

- (i) administering a water insoluble nanoparticle having at least one neutron capture element in an inorganic form to an individual;
- 10 (ii) allowing said nanoparticles to accumulate at a desired location in the body; and
- (iii) administering neutrons to said individual.

15 38. A method of claim 37 for the treatment or ablation of cancer or other diseased tissues e.g. lymphomas, skin cancer, breast cancer, lung cancer, head and neck cancer, bone cancer, prostate cancer, cancer of the pancreas, cervical cancer, brain cancers e.g. glioblastomas, primary and secondary metastases and benign and metastatic prostate cancers, e.g. benign prostate hyperplasia.

20

39. A method as claimed in claim 38 wherein said tumour is solid and discrete.

25 40. A method as claimed in claim 38 or claim 39 further comprising the step of removing a tumour by surgery.

41. A method as claimed in any of claims 38 to 40 further comprising analysing the concentration of the neutron capture element in the target tissue.

30

42. A method as claimed in claim 41 wherein said analysis comprises MRI, PET or SPECT imaging.

43. The use of water insoluble nanoparticles comprising at least one neutron capture element in an inorganic form in a method for neutron capture therapy.

5 44. A use as claimed in claim 43 for the treatment or ablation of cancer or other diseased tissues e.g. lymphomas, skin cancer, breast cancer, lung cancer, head and neck cancer, bone cancer, prostate cancer, cancer of the pancreas, cervical cancer, brain cancers e.g. glioblastomas, primary and secondary metastases and benign and metastatic prostate cancers, e.g.
10 benign prostate hyperplasia.

45. A use as claimed in claim 44 wherein said tumour is solid and discrete.

46. A process for the preparation of water insoluble nanoparticles
15 comprising at least one neutron capture element in an inorganic form, said process comprising:
(i) providing at least a first mass of said neutron capture element in an inorganic form;
(ii) providing at least a second mass of the same type of material;
20 (iii) mixing said first and second masses in the absence of other abrasive material;
(iv) causing frictional abrasion between said first and second masses; and
(v) collecting said nanoparticles.

25 47. A process as claimed in claim 46 wherein at least one further, non abrasive material is included in said mixing step (iii).

48. A process as claimed in claim 47 wherein said further, non abrasive material is selected from the group consisting of vanadium (V), manganese
30 (Mn), iron (Fe), ruthenium (Ru), technetium (Tc), chromium (Cr), platinum (Pt), cobalt, (Co), nickel (Ni), copper (Cu), zinc (Zn), germanium, (Ge), indium (In), tin (Sn), yttrium (Y), gold (Au), barium (Ba), tungsten (W), and gadolinium (Gd).

49. A process as claimed in any of claims 46 to 48 wherein the resulting nanoparticles are characterised by any of the features of claims 2 to 10 or claims 12 to 27.

5 50. A water insoluble nanoparticle substantially as hereinbefore described.

51. A pharmaceutical composition substantially as hereinbefore described.

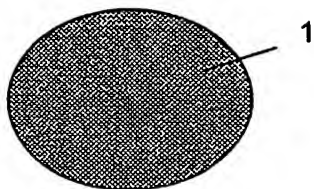
10 52. A method for neutron capture therapy substantially as hereinbefore described.

53. A process for the preparation of water insoluble nanoparticles substantially as hereinbefore described.

15

Figure 1

A



B

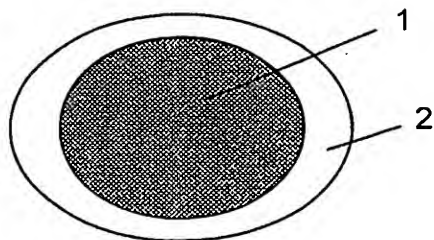


Figure 2

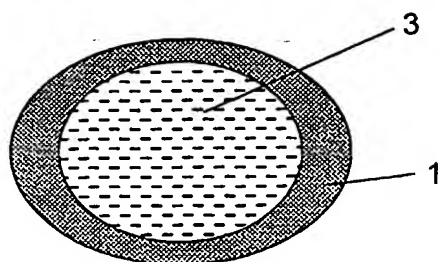


Figure 3

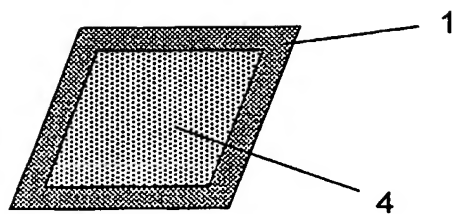
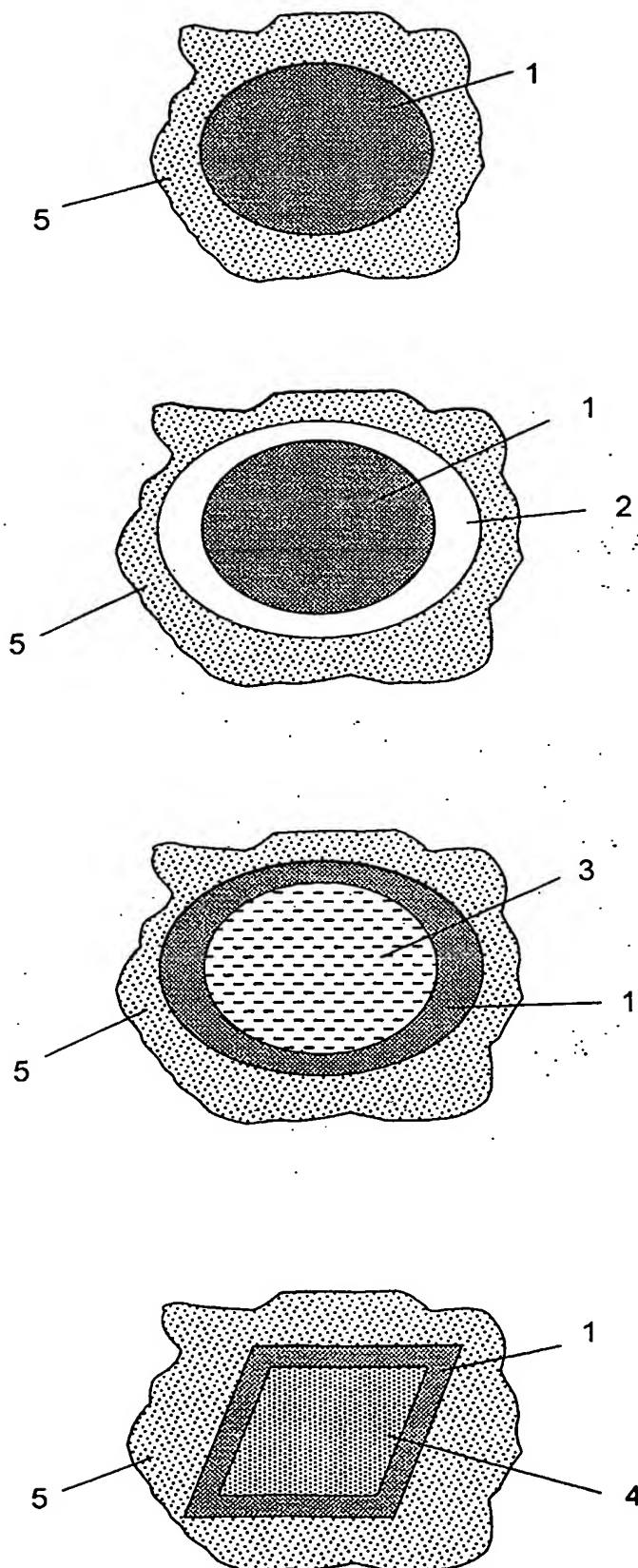


Figure 4



INTERNATIONAL SEARCH REPORT

Int. Application No
PCT/GB 02/05927A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K41/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EMBASE, BIOSIS, CHEM ABS Data, EPO-Internal, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01 34196 A (BRANDSCH MATTHIAS ;FRANK WILLY (DE); ARNOLD MANFRED (DE); FLEIG WO) 17 May 2001 (2001-05-17) claims 1,5,10,14,16-18 ---	1-53
X	WO 00 44682 A (UNIV MISSOURI) 3 August 2000 (2000-08-03) claims 1,8,9 --- -/--	1-53

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- * & * document member of the same patent family

Date of the actual completion of the international search

26 March 2003

Date of mailing of the international search report

04/04/2003

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Berte, M

INTERNATIONAL SEARCH REPORT

Int. Application No
PCT/GB 02/05927

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TOKUMITSU H ET AL: "CHITOSAN-GADOPENTETIC ACID COMPLEX NANOPARTICLES FOR GADOLINIUM NEUTRON-CAPTURE THERAPY OF CANCER: PREPARATION BY NOVEL EMULSION-DROPLET COALESCENCE TECHNIQUE AND CHARACTERIZATION" PHARMACEUTICAL RESEARCH, NEW YORK, NY, US, vol. 16, no. 12, 1999, pages 1830-1835, XP000951445 ISSN: 0724-8741 abstract; figure 1	1
X	WO 00 45826 A (UNIV MISSOURI) 10 August 2000 (2000-08-10) page 4, line 30 -page 5, line 1; claims 1,24,25	1
X	LAWACZECK R ET AL: "GADOLINIUM NEUTRON CAPTURE THERAPY (GDNCT) WITH MRI CONTRAST MEDIA IN VITRO STUDIES" CANCER NEUTRON CAPTURE THERAPY, XX, XX, 1996, pages 859-864, XP000951499 abstract	1,37-42
X	US 5 443 813 A (HAINFELD JAMES F) 22 August 1995 (1995-08-22)	1-53
Y	column 7, line 49 - line 51; claims 1,5 column 8, line 42 - line 59 column 9, line 4 - line 20 column 10, line 9 - line 11	1-53
X	DATABASE CA 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; FUKUMORI, YOSHINOBU ET AL: "Nano-particulate design and preparation for targeting and controled release of drugs" retrieved from STN Database accession no. 135:127058 XP002224876 abstract & INTERNATIONAL CONFERENCE ON PROCESSING MATERIALS FOR PROPERTIES, PROCEEDINGS, 2ND, SAN FRANCISCO, CA, UNITED STATES, NOV. 5-8, 2000 (2000) 453-458. EDITOR(S): MISHRA, BRAJENDRA;YAMAUCHI, CHIKABUMI. PUBLISHER: MINERALS, METALS & MATERIALS SOCIETY, WAR,	1-53

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INTERNATIONAL SEARCH REPORT

In
PCT/GB 02/05927

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	THOMAS J. ET AL.: "Dodeca(carboranyl)-substituted closomers: toward unimolecular nanoparticles as delivery vehicles for BNCT." CHEM COMMUN (CAMB), (2001 SEP 21) (18) 1884-5., XP002224874 abstract	1-53
Y	ALI O. SEZER ET AL.: "Chemical vapor deposition of boron carbide." MATERIALS SCIENCE AND ENGINEERING, vol. B79, 2001, pages 191-202, XP002224875 abstract	1-53
A	MACPHERSON M S ET AL: "DOSE DISTRIBUTIONS AND DOSE RATE CONSTANTS FOR NEW YTTERBIUM-169 BRACHYTHERAPY SEEDS" MEDICAL PHYSICS, AMERICAN INSTITUTE OF PHYSICS. NEW YORK, US, vol. 22, no. 1, 1995, pages 89-96, XP000505147 ISSN: 0094-2405 abstract	
A	CHENG ET AL: "Neutron-activatable glass seeds for brachytherapy" JOURNAL OF NUCLEAR MEDICINE, SOCIETY OF NUCLEAR MEDICINE. NEW YORK, US, vol. 35, no. 5, 5 June 1994 (1994-06-05), page 242P XP002111090 ISSN: 0161-5505 abstract	

INTERNATIONAL SEARCH REPORT

ational application No.
PCT/GB 02/05927

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 37-41, 52 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

Continuation of Box I.2

Present claims 1-53 relate to an extremely large number of possible products. In fact, the claims contain so many options or variables that a lack of clarity (and conciseness) within the meaning of Article 84 EPC arises to such an extent as to render a meaningful search of the claims impossible. Consequently, the search has been carried out for those parts of the application which do appear to be clear (and concise), namely products have been searched recited in the examples.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

In International Application No

PCT/GB 02/05927

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			DE 19953637 A1	23-05-2001
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